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14. ABSTRACT
The New York Structural Biology Center (NYSBC), a leading center in magnetic resonance spectroscopy and X-ray crystallography, has
worked with researchers at Albert Einstein College of Medicine (AECOM), and scientists at the Edgewood Chemical Biological Center
(ECBC) in several projects whose objective is the analysis of the chemical and biological makeup of warfare

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Report Title

Agents Within our Midst

ABSTRACT

The New York Structural Biology Center (NYSBC), a leading center in magnetic resonance spectroscopy and X-ray crystallography, has worked with researchers at Albert Einstein College of Medicine (AECOM), and scientists at the Edgewood Chemical Biological Center (ECBC) in several projects whose objective is the analysis of the chemical and biological makeup of warfare agents. The projects include: the structural analysis of Staphylococcal Enterotoxin B (SEB), considered to be a potential biological threat with particular potency on the battlefield; the analysis of recognition molecules for use in automated detection devices that are sensitive to biological toxins that could be deployed in public places; the identification of genes that are candidate targets for potential drugs to counteract pathogens that could be used as warfare agents; the reactions of man made surfaces to chemicals used for decontaminating after exposure to chemical warfare agents; and the development of bio-monitoring protocols for civilian and service personnel during a chemical attack. These efforts have resulted in greater knowledge about the behavior of biological and chemical warfare agents in the environment. This knowledge will help facilitate their detection and the design and development of countermeasures for application to battlefield defense and homeland security.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received	Paper
2012/03/14 0 3	Boris Itin, George W. Wagner. Comment on “27Al, 47,49Ti, 31P, and 13C MAS NMR Study of VX, GD, and HD Reactions with Nanosize Al2O3, Conventional Al2O3 and TiO2, and Aluminum and Titanium Metal”, Journal of Physical Chemistry C, (07 2008): 0. doi: 10.1021/jp802847m
2012/03/14 0 2	G.W. Wagner, L.R. Procell, S. Munavalli. 27Al, 47,49Ti, 31P, and 13C MAS NMR Study of VX, GD, and HD Reactions with Nanosize Al2O3, Conventional Al2O3 and TiO2, and Aluminum and Titanium Metal, Journal of Physical Chemistry C, (11 2007): 0. doi: 10.1021/jp074511k
2012/03/14 0 4	George W. Wagner, Roderick A. Fry. Observation of Distinct Surface AlIV Sites and Phosphonate Binding Modes in ?-Alumina and Concrete by High-Field 27Al and 31P MAS NMR, The Journal of Physical Chemistry C, (07 2009): 0. doi: 10.1021/jp902474z
2012/03/14 0 5	Gregory W. Peterson, George W. Wagner, Alex Balboa, John Mahle, Tara Sewell, Christopher J. Karwacki. Ammonia Vapor Removal by Cu3(BTC)2 and Its Characterization by MAS NMR, The Journal of Physical Chemistry C, (08 2009): 0. doi: 10.1021/jp902736z
2011/08/31 1 1	A. K. Varshney, X. Wang, E. Cook, K. Dutta, M. D. Scharff, M. J. Goger, B. C. Fries. Generation, Characterization, and Epitope Mapping of Neutralizing and Protective Monoclonal Antibodies against Staphylococcal Enterotoxin B-induced Lethal Shock, Journal of Biological Chemistry, (01 2011): 0. doi: 10.1074/jbc.M110.212407

TOTAL: 5

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

2007
Lisa M. Reilly1, Vicky L.H. Bevilacqua1, Jeffrey S. Rice1, Michael J. Goger2, Bernard J. Benton1, Boris Itin2, and Stephen Huhn3
"Metabolomic Study on the Effects of VX on Brain Tissue in the Rat"
1 Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD 21010-5424
2New York Structural Biology Center, 89 Convent Way, New York, NY 10027-755
3 Wyeth Pharmaceuticals, 200 Cambridgepark Dr., Cambridge, MA 02140
Poster Presentation: Chemical and Bologial Defense Science and Technology Conference, Nov. 2007

2008
Lisa M. Reilly1; Vicky L. H. Bevilacqua1; Jeffrey
S. Rice1; Michael J. Goger2; Bernard J. Benton1; Boris Itin2;
Steve Huhn3;
"1H NMR Metabolic Response of Rats to Nerve Agent Exposure"
1Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD; 2New York Structural Biology Center, New York, NY; 3Wyeth Pharmaceuticals,Cambridge, MA
Poster presentation, 49th ENC, March 9 - 14, 2008
Asilomar Conference Center, Pacific Grove, CA

2009
Wagner GW, Peterson GW, Balboa A, Mahle J, Sewell T, Karwacki CJ,
"NMR Studies of the Metal-Organic Framework CU3BTC2"
Poster presented at 50th ENC, Asilomar CA, 3/29 - 4/5/2009

Bettina Fries (Albert Einstein College of Medicine)
"Treatment of staphylococcal enterotoxin B (SEB) induced lethal shock
with specific monoclonal antibodies."
Presentation at Northeast Biodefence Center Annual Meeting 9/2/2009.

Avanish K. Varshney1*, Xiaobo Wang1, Kaushik Dutta2, Matthew Scharff3, Michael J. Goger2 and Bettina C. Fries1
"TREATMENT OF STAPHYLOCOCCAL ENTEROTOXIN B (SEB) INDUCED LETHAL SHOCK WITH SPECIFIC MONOCLONAL ANTIBODIES (mAb) Therapeutics"
1Department of Medicine, Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, 2New York Structural Biology Center, New York, and 3Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY, USA
Poster Presentation at Northeast Biodefence Center Annual Meeting 9/2/2009.

2010
Avanish K. Varshney*1,2, Xiaobo Wang1,2, Kaushik Dutta3, Matthew Scharff4, Michael J Goger3 and Bettina Fries1
"NEUTRALIZATION OF STAPHYLOCOCCAL ENTEROTOXIN B (SEB) INDUCED LETHAL SHOCK AND EPITOPE MAPPING OF SEB SPECIFIC MONOCLONAL ANTIBODIES"
1Department of Medicine, 2Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY; 3New York Structural Biology Center, NY, 4Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY
Poster Presentation atNortheast Biodefence Center NIAID Region II Center of Excellence for Biodefense and Emerging Infectious Diseases
Mohonk Mountain House, New Paltz, NY, November 1-3, 2010

2011
Kaushik Dutta3, Avanish K. Varshney1,2, Xiaobo Wang1,2, Michael J. Goger3 and Bettina C. Fries1,2
"NMR Epitope Mapping of SEB Specific Monoclonal Antibodies"
1Department of Medicine, 2Microbiology and Immunology, at Albert Einstein College of Medicine, Bronx, New York, NY 10461 and 3New York Structural Biology Center, New York, NY 10027
Poster presentation at Keystone Symposia:Frontiers of NMR in Biology (A3)Big Sky MT,Jan 8 - 13, 2011

Number of Presentations: 7.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Michael Goger	0.35	
Kaushik Dutta	0.35	
FTE Equivalent:	**0.70**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

ARO Final Report
"Agents Within Our Midst"
W911NF-07-1-0053

High-field Nuclear Magnetic Resonance (NMR) and X-ray crystallography afford tremendous capability for the study of atomic-level structural characterization of chemical and biological compounds. This grant provided access for researches at Edgewood Chemical Biological Center (ECBC) to the state of the art structural biology facility at New York Structural Biology Center (NYSBC). NYSBC is a consortium of 9 preeminent medical schools and universities and is a leading center in magnetic resonance spectroscopy and X-ray crystallography. Multiple projects important to homeland defense at ECBC, ranging from structural studies to provide protection from exposure to Staphylococcal Enterotoxin B (SEB) to the decontamination of solid surfaces exposed to nerve agents, benefited from the instrumentation and technical expertise provided by NYSBC under this program.

***Analysis of Staphylococcal Enterotoxin B*:** (NYSBC Task PI - Michael Goger; AECOM Task PI – Bettina Fries;)

Staphylococcal Enterotoxin B (SEB) is one member of a large class of enterotoxins produced by staphylococcal bacteria that is and is classified as a CDC select agent which has the potential to be used as a biological weapon.[1] Exposure to SEB, a superantigen that triggers cytokine production and T-cell proliferation can result in SEB induced lethal shock (SEBILS), characterized by profound emisis, hypotension and multi-organ failure. In addition, MSRA strains of staphylococcal are becoming an increasingly significant problem in hospital settings. Currently there are no prophylactic treatments or antidotes to prevent the harmful effects of exposure to SEB. For these reasons research in the development of treatment of and protection against SEB is an active area of study. Our collaborator, Dr. Bettina Fries, has developed monocolonal antibodies (mAb), designated 20B1, 14G8, and 6D3, against SEB[2] which have been shown to mitigate the mitogenic effects of SEB *in vitro* and are protective against SEBILS in the BALB/c mouse model system.[3] However, when tested in the more sensitive HLA-DR3 transgenic mouse model[4], only 20B1 was found to be protective against SEBILS. Interestingly, even though 14G8 and 6D3 were not effective alone, when administered simultaneously in the HLA-DR3 mice protection against SEBILS was achieved. Additionally, it has been shown through ELISA based epitope mapping, that each of the 3 mAbs bind independently to SEB and that the C-terminal residues of SEB are important for the binding of each of the mAbs. Removal of these residues destroys the ability of the mAbs to bind SEB.[4] These ELISA based methods provided a preliminary indication of the binding epitope but are not detailed enough of to provide information that would be useful for the further improvement of these mAbs as prophylactic treatment of SEB.

We have focused on using structural biology methods to more precisely define the binding epitope on SEB for these protective antibodies. The improved understanding of the interaction between the mAbs and SEB resulting from this study will provide insight into the protective mechanism of the mAbs, which will be applied to improving the binding, and neutralizing characteristics of the mAbs. The first of these approaches that we employed is NMR, a powerful technique for studying protein-protein interactions in solution. The other approach is x-ray

crystallography, a solid-state technique that excels in providing detailed structures of biological macromolecules.

The ultra high-field NMR spectrometers available at NYSBC were used to study the interaction between the neutralizing mAbs and SEB to better define the binding surface of SEB responsible for binding the mAbs. NMR chemical shift perturbation titrations with Fab (fragment, antigen binding regions) domains of 20B1, 14G8, and 6D3 using deuterated (^{2}H) SEB. The Fab fragments retain all of the binding capabilities as the parent antibodies bur because of their smaller size they are much more amenable to NMR studies. The deuteration of SEB necessary to reduce the unfavorable relaxation properties that are associated with the large SEB:Fab complexes. Several ratios of SEB:Fab complexes were prepared and both ^{1}H-^{15}N transverse relaxation-optimized spectroscopy (TROSY)[8] and ^{1}H-^{15}N cross-correlated relaxation-enhanced polarization transfer (CRINEPT)[7] experiments were recorded.

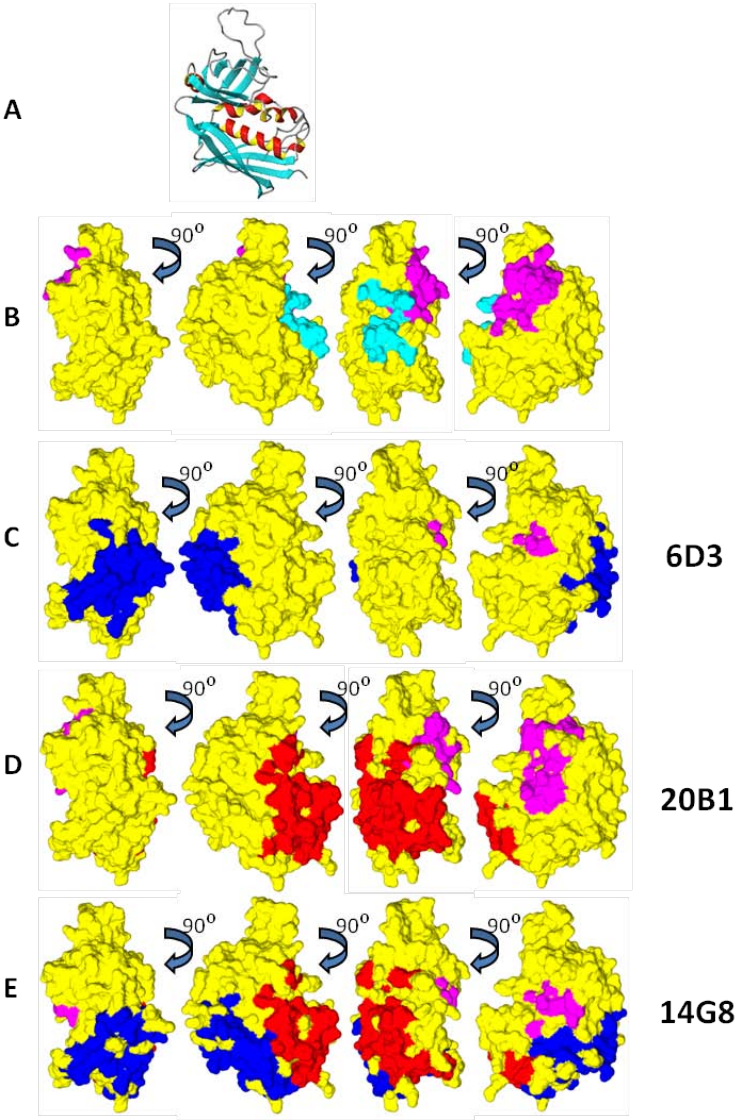


Figure 1. TCR and MHC binding surfaces of SEB (pdb:seb3)[6]. (A) The ribbon structure illustrates that SEB can be divided into two domains, and is shown in the same orientation as the surface illustration directly below. (B) On the surface illustrations MHC binding site is colored cyan, and TCR binding site is colored purple. Binding surfaces identified from the analysis of the TROSY/CRINEPT data: (C) 6D3 shown in blue, (D) 20B1 shown in red and (E) 14G8 shown in blue are residues common with 6D3 and in red are residues common with 20B1.

In the analysis of the TROSY/CRINEPT experiments there are two potential observations to consider. The first is if the resonance is present, at the same chemical shift, in both the TROSY and CRINEPT, and is interpreted as that residue is unaffected and not involved in the complex. The second observation is that there is a chemical shift difference (CSD) between the two spectra such that the resonance is present in the TROSY but absent the CRINEPT. In this case the interpretation is that the residue is affected in the complex and that its' resonance position has been shifted as a result of complex formation due to a direct interaction with the Fab, or alternatively because of a change in the SEB conformation as a result of binding the Fab. This analysis has been carried out for each observable cross-peak in the TROSY spectrum for each of the three SEB/Fab complexes and producing a list of the residues of SEB that are affected by the binding of the

Fabs to SEB. The crystal structure of SEB is colored according to these residues to indentify the binding surface of SEB for the Fabs (Figure 1). Interestingly it was found that each individual Fab has a distinct binding surface that is different from active receptor binding sites of SEB. Additionally, the binding surfaces identified in Figure 1 are consistent with data from the Fries laboratory that shows that any two of the three antibodies can bind simultaneously to SEB.

At the outset of this research one of the goals was to determine if the antibodies developed in the Fries laboratory recognized the same binding surfaces (Figure 1, panel B) used by the T-cell receptor (TCR) and major histocompatability complex (MHC) receptor that are responsible for the toxic effects of SEB. In other words, do the antibodies provide their protective effects by binding directly to the same SEB sites as the receptors or do the antibodies bind at sites remote from the receptor binding sites and provide protection via mechanisms other than direct competition. The observations in the pattern of Fab binding surfaces argue against the direct competition model. However, there does seem to be some evidence, at least for 20B1 (Figure 1, panel D), that the binding of the antibody may alter the conformation of the receptor binding sites. In the case of 20B1, we observe that there are CSDs (colored purple in panel D) in the region of the MHC and TCR receptor binding sites. While these residues are not contiguous with the rest of the binding surface, it is possible that these CSDs result from conformational changes in this region of SEB due to the binding of 20B1. These conformational changes could then render SEB incapable of binding the MHC and TCR receptors. This hypothesis needs to be confirmed by additional studies.

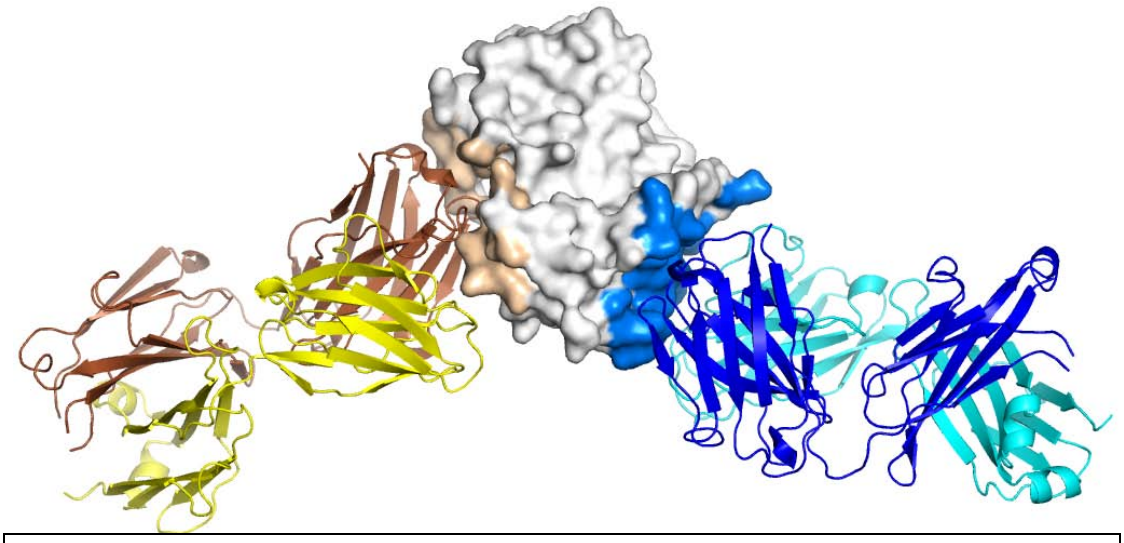


Figure 2: Composite model structure of triplex SEB:14G8:20B1. Thjs model was created from individual crystal structures of separate SEB:20B1 and SEB:14G8 complexes. Each structure identifies a different binding surface on SEB for the Fab. These surfaces do not overlap and are consistent with the proposal that any 2 mAbs can bind simultaneously to SEB.

While the initial NMR analysis was helpful in identifying the binding epitopes on SEB for the three antibodies, a model of the SEB/Fab complex would be more informative. In order to confirm the indentified binding surfaces and to generate a more detailed structure of the SEB;Fab complexes x-ray crystallography was initiated. Crystals of SEB complexes with 20B1 and 14G8 have been generated. Initial attempts to produce a crystal of the SEB:6D3 complex were

unsuccessful and are being repeated. The crystals structures of the SEB:20B1 and SEB:14G8 complexes have be solved to a resolution <3A. These structures are in general agreement with the binding surfaces indentified by NMR. Importantly, they confirm that these two Fabs do indeed bind different surfaces in a manner that would not preclude them from binding simultaneously (Figure 2). With this promising result, trails to produce a crystal of the triplex of SEB:20B1:14G8 were initiated. A crystal was produced that diffracts to ~2.6A, however the data set was not complete. Additional data will need to be collected to solve the structure, however it does confirm that 2 Fab fragments are bound SEB simultaneously. Work on this project will continue at NYSBC beyond the end of this grant.

Structural Studies of Flexible Molecules: (ECBC Task PI – Terry Henderson).

There is tremendous interest in developing automated detection devices for the continuous monitoring of high-risk areas such as airports and shopping malls. Recognition molecules for these devices must be very robust and capable of monitoring complex environmental samples over long periods of time, without significant loss of activity. Dr. Henderson is exploring the use of small, synthetic molecules with large degrees of conformational flexibility as ligands for designing a new generation of robust biosensors. His approach uses extensive nuclear magnetic relaxation (NMR) spectroscopy and computer modeling to examine the conformational changes in the ligands, and the associated thermodynamic changes, upon binding to the surfaces of protein toxin targets. The prototype binding reaction for this effort is that of the anti-tumor agent doxorubicin (580 grams/mole) to the tetanus toxin surface.[9]

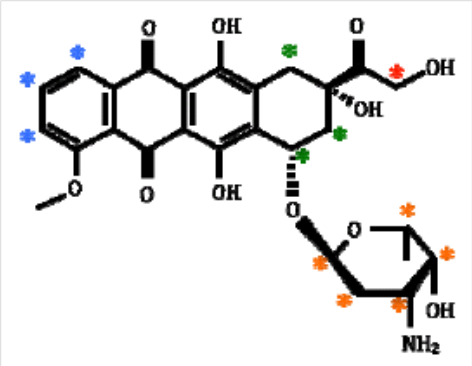


Figure 5. Flexibility of Doxorubicin by atom, *>*>*>*

Dr. Henderson collected all high field NMR experiments with doxorubicin necessary for this project at NYSBC. $^{13}C\{^{1}H\}$ relaxation times and NOE enhancements for the 12 doxorubicin carbon atom sites have been conducted at two field strengths, representing ^{13}C resonance frequencies of 201 and 226 MHz. Additionally, ^{13}C spin lattice relaxation times have been measured for the sites at 175 MHz, giving a total of 84 individual measurements for data interpretation. This interpretation is focused on understanding the details of the solution conformational behavior experienced at the carbon atom sites, especially the explicit types of motions involved (methyl group rotation and movements of large segments of the doxorubicin, for example) and their absolute time scales. Initial results suggest that the conformational behavior of doxorubicin in solution is a composite of a very rigid aromatic ring system, limited ring librations for the cyclohexane ring, fast librations for the carbohydrate group, and even faster motions for the pendant group. Early stages of modeling the doxorubicin-toxin binding interaction suggest that the flexibility of the carbohydrate and pendant groups are important for adopting conformations to optimize binding, while the rigid aromatic ring provides a hydrophobic framework to hold these flexible groups together and steer them toward the toxin hydrophobic pocket. Further modeling and refinement of the binding event should give some insight into the thermodynamics changes involved. Together, the data will be used to refine and finalize the

computer experiments modeling the doxorubicin-tetanus toxin binding process and associated thermodynamics.

Solid State Nuclear Magnetic Resonance of Surface Interactions: (ECBC Task PI – George Wagner)

The results of this basic research will enable a better understanding of the detoxifying reactions of chemical warfare agents (CWAs) and toxic industrial chemicals (TICs) on reactive, decontaminating sorbents, filtration media, and/or environmental matrices. This knowledge will facilitate the design of enhanced sorbent decontaminants/filtration media as well as the development of improved environmental restoration and/or remediation procedures to be implemented in the advent of a CWA attack.

Decontaminating sorbents are typically fine powders that soak up and destroy CWAs neutralizing their threat. Dr. Wagner has focused on reactions of metal oxides, alumina and titania, with organophosphorous based CWAs by ^{27}Al, ^{47,49}Ti, ^{31}P, and ^{13}C MAS NMR. Access to ultra-high field (750 and 900MHz) solid state NMR spectrometers at NYSBC has been valuable in characterizing reaction products of chemical warfare agents and decontaminating sorbents. As stated in earlier reports these previous studies have resulted in four manuscript publications.[10-13] In particular, Dr. Wagner was able to demonstrate that ^{27}Al, ^{47,49}Ti MAS spectra obtained at ultra-high field provided unprecedented resolution when compared to spectra obtained at lower field. In the case of CWAs reacting with γ-Al2O3 (Selexsorb CDX, Alcoa) ^{27}Al MAS spectra obtained at 21.1T afforded the observation of *hydroxylated* γ-Al2O3 surfaces, enabling resolution of distinct bulk and surface Al_{IV} sites. Some of the resolved Al_{IV} sites are sensitive to surface hydroxylation/dehydroxylation are attributed to surface Al_{IV}-OH groups (apparently having been observed for the first time). Although the number of surface Al_{IV} sites detected by high-field ^{27}Al MAS NMR (three) is in agreement with current surface models, their dehydroxylation behavior does not entirely concur with proposed dehydroxylation mechanisms.

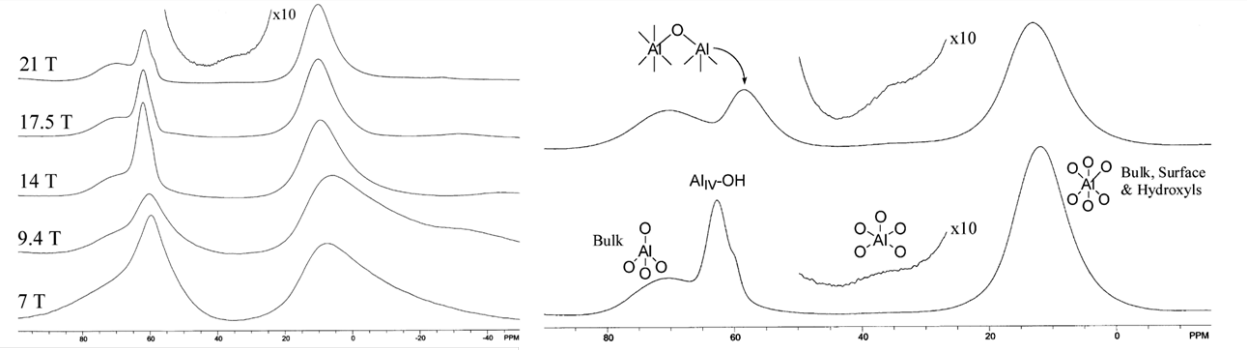


Figure 3: Right Panel. 27 Al MAS NMR spectra obtained for g-Al2O3 at 7, 9.4, 14, 17.5 and 21 T. The inset shows enhanced (10x) Alv region for the 21 T spectrum. Left Panel. 21 T spectra obtained for as-received (hydrated, bottom) and partially dehydrated (400 C, top) g-Al2O3 illustrating assignments of aluminum oxides.

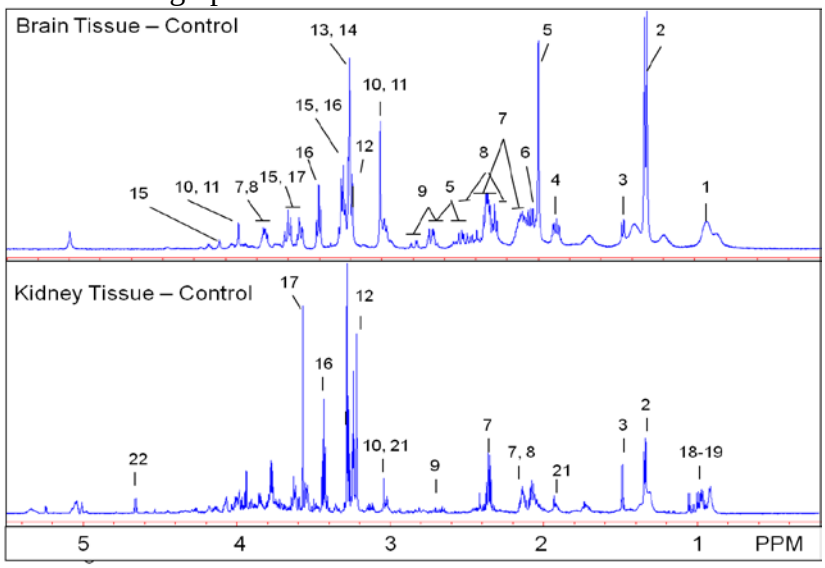
Biomarkers of Agent Exposure: (ECBC Task PI – Dr. Vicky Bevilacqua)

Adequate protection of troops and civilians against chemical warfare agent (CWA) attack, depends on methods of monitoring for exposure-related physiological effects (bio-monitoring). Current detection methods rely on the assessment of cholinesterase activity in blood by quantization of bound agent in blood[14], and detection of the agents' metabolites in urine and blood.[15,16] These methods are focused on the detection of the agent itself and a limited number of possible metabolites. Subtle effects that occur at very low level of exposure, prior to obvious external symptoms and detection by current methods, are of particular interest for protection measures. Such effects may be signaled by changes in concentrations of endogenous metabolites (biomarkers) that have not traditionally been investigated. This project explores the use of high field nuclear magnetic resonance in combination with principal component analysis (NMR-PCA) as a means of identifying biomarkers of CWA exposure in animal models. The NMR-PCA procedure allows simultaneous analysis of multiple metabolites. The biomarkers identified as part of this project could be monitored during exposure (serum) or post-exposure (serum, brain tissue, kidney tissue) in evaluation of protection measures such as skin creams or protective clothing employing animal models. The data obtained as part of this study could also be combined with similar future studies on multiple animal models for an improved time-dependent mathematical human physiological model of agent exposure. Physiological models are important in simulation of CWA attacks on a large scale as part of hazard mitigation planning.

Male Sprague-Dawley rats were exposed to VX vapor by whole body inhalation in an isolated chamber (0.133-0.154 mg/m^3 per minute for 60 or 240 min) to simulate a CWA attack. Serum samples were collected at 0 hr, 1 hr, 24 hr, and 168 hr post exposure and tissue samples were collected 14 days post-exposure. NMR samples from the test animals including blood serum, brain and kidney tissues were shipped to NYSBC for data collection. Three separate ^1H 1D HR-CPMAS NMR experiments were carried out using the 750 MHz spectrometer for each the brain and kidney tissues samples. Serum samples were analyzed by 1D CPMG[17] spin-echo pulse sequence using the 900 MHz NMR spectrometer at NYSBC. Following the collection of the spectra, each spectrum was integrated over 256 individual 0.04ppm segments (bins), excluding the water region, as preparation for the principle component analysis (PCA). PCA is a statistical method that calculates a set of descriptors, the principal components (PCs), that are linear combinations of the original variables, in this case the integrated values of the bins, with each PC orthogonal to the others. PC1, the first PC, explains the most variance in the data set while each successive PC explains the most variance remaining after the previous PC. PCA is an efficient method of analysis for comparing subtle differences in related complicated data sets by plotting PC1 vs PC2 where data from one set groups in a region of the plot separated from the group of the second data set.

The PCA of the serum samples collected at time points as described above appear to plot the trajectory of metabolic changes in agent exposed animals as seen in figure 5. The trajectory exhibits a maximum change at 1-hour after exposure and a return to baseline values by 168 hours after exposure. A metabolite in the glucose and amino acid alpha proton region (3.72 ppm) is responsible for this separation. These results are very suggestive that metabolic changes as a result of VX-exposure can be monitored, it is acknowledged that these changes could also be the result of stress experienced by test animals. Comparison of serum samples from control animals will be conducted to confirm these observations.

Similar analysis of the brain tissue of the VX-exposed animals showed metabolic profile changes that are characterized by increased lactate, N-acetylaspartate, glutamate, phosphorylcholine/ glycerophosphorylcholine, and decreased creatine/phosphorcreatine spectral regions. Whereas, the kidney samples exhibited VX-correlated changes that included increases in leucine, isoleucine, lactate, and taurine and decreases in choline and valine. In each case the tissues samples were collected 14-days post-exposure, and are indicative that alterations in the metabolic fingerprints of these tissues can indeed be observe as a result of VX-exposure.



1. Lipids, LDL/HDL, CH_3
2. Lactate
3. Alanine
4. Acetate
5. N-acetylaspartate
6. N-acetylaspartylglutamate
7. Glutamte
8. Glutamine
9. Aspartate
10. Creatine
11. Phosphocreatine
12. Choline
13. Phosphorylcholine
14. Glycerophosphorylcholine
15. myo-Inostol
16. Taurine
17. Glycine
18. Isoleucine
19. Leucine
20. Valine
21. Lysine
22. B-Glucose

Figure 4: Expanded regions of ^{1}H CPMG spectra for rat brain tissue (top, 2ms total spin relaxation delay, 500 MHz) and for rat kidney tissue (bottom, 120 ms total spin-relaxation delay, 750 MHz). The assignments of selected metabolites are based on literature values.[18,19] The broad peaks in the brain tissue spectrum in the 0.7 to 1.4 ppm region are due to lipid that have not been completely edited out by the short CPMG relaxation delay.

The results from these studies are encouraging that the combined NMR-PCR approach can be used biomonitoring of nerve agent exposure. The data obtained as part of this study could also be combined with similar future studies on multiple animal models for an improved time-dependent mathematical human physiological model of agent exposure. Physiological models are important in simulation of CWA attacks on a large scale as part of hazard mitigation planning. Additionally, the identification of these biomarkers could potentially lead to a quick multi-metabolite test for VX vapor exposure, allowing for prevention of secondary exposure and for use as a forensic tool. However, additional studies will be required to confirm these potential applications.

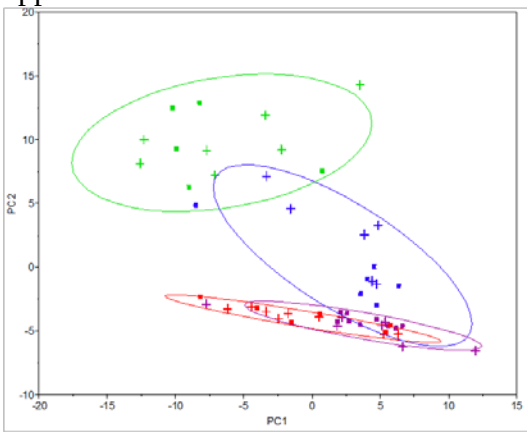


Figure 5: PC2 vs. PC1 scores plot for two serum sample sets with same total agent exposure. Red, pre-exposure; Green, 1 hr. post-exposure; Blue, 24 hrs. post-exposure; Purple, 168 hrs. post-exposure. Animals were exposed to the same total amount of VX for either 60 min (square) or 240 min (cross). The plots shows the different scores plot locations of 1th ^{1}H spectra obtained at various time points post-exposure. The results appear to show a metabolite at a chemical shift of 3.72 pp is responsible for the separations observed in the trajectory.

Structural Analysis of Biodefense Targets (AECOM Task PI- Steve Almo).

There is an essential need to identify and characterize new molecular targets for the development of novel therapeutic approaches to combat the threats of bioterroism. The National Institute of Allergy and Infectious Diseases (NIAID) has defined the Category A pathogens, including Cacillus anthracis (anthrax), Yershinia pestis (plague), Variola major (small pox), and Fancisella tularensis (tularemia) as the microorganisms that pose the greatest threat for the use as agents of bioterrorism. Through informatics analysis, Dr. Almo has identified hundreds of genes that are candidate targets for the development of drugs to combat the Category A-C Pathogens. The focus has been on three related sets of targets; **1**) orthologs of essential genes in nucleoside and nucleotide metabolism, including nucleoside phosphorylases, nucleoside hydrolases and phosphoribosyl transferases, **2**) orthologs of the targets of FDA-approved drugs and **3**) secreted proteins from the Category A-C Pathogens and their orthologs). These classes of proteins are of particular importance for understanding pathogen biology and for the potential development of new therapeutics. The study of essential genes (**1**), and especially those related to already validated targets of FDA-approved drugs (**2**), provide a potentially rapid avenue to develop new therapeutics. The study of secreted pathogen proteins is very likely to define new biology, as genome economy is a major evolutionary pressure, and these secreted proteins are conserved to evade host immune responses and to co-opt host signaling pathways.

Enzymes targets from *Salmonella*, *Yersinia*, *Vibrio*, *B. anthrax* and *F. tularensis* were identified, cloned into *E. coli* expression vectors, expressed and purified to homogeneity. Dr. Almo cloned 672 genes into both pET 30 AND pET23. The use of two different pET vectors is of critical importance, as in our hands this leads to a nearly 50% increase in the number of soluble proteins. We have performed small-scale expression testing the 576 pET30 constructs, evaluated 96 of these in large scale fermentations and successfully purified 35 (Figure 6). Of these eight high-resolution X-ray structures have been determined (Figure 7), including the structures of *Bacillus anthracis* and *Vibrio cholera* hypoxanthine phosphoribosyl transferase (HPRT) bound to the transition state inhibitor Immucillin-G (Figure 8).

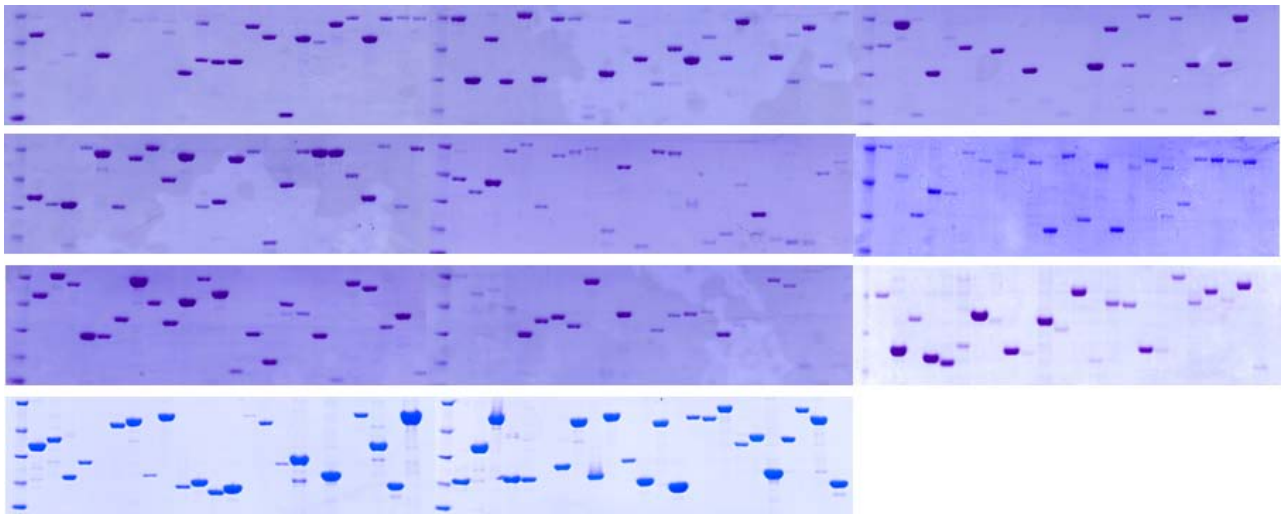


Figure 6: High-throughput small-scale purification of proteins and orthologs from Category A-C bacterial pathogens. Illustrated are 275 orthologs of FDA-approved drug targets from Category A-C pathogens purified using either N or C-terminal His_6-Strep affinity tags.

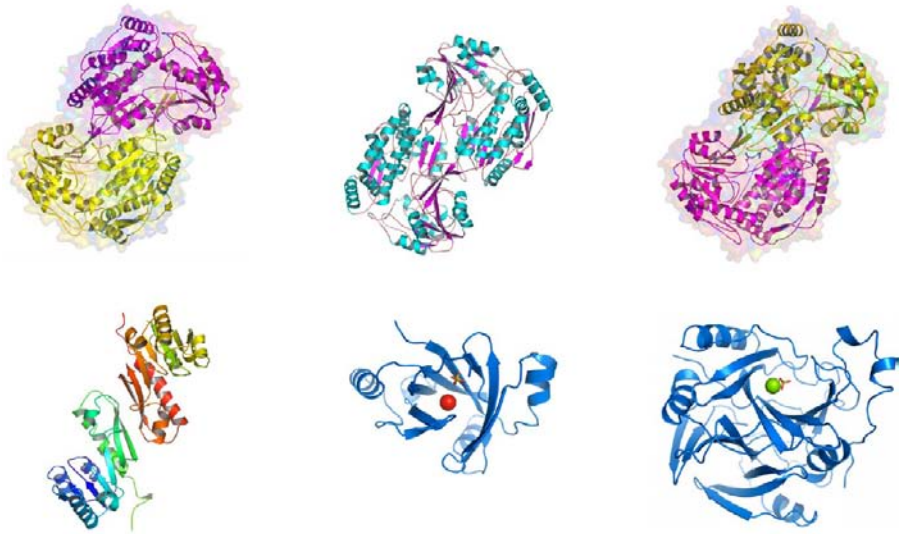


Figure 7: Six of the eight structures of pathogen orthologs of FDA-approved drug targets.

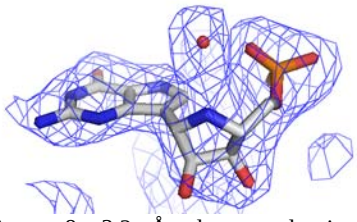


Figure 8. 3.2 Å electron density for Immucillin-G bound in the catalytic site of *Bacillus anthracis* hypoxanthine phosphoribosyl transferase (contoured at 1σ).

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